NOVEL ANALOGS OF VASOACTIVE INTESTINAL PEPTIDE

Vasoactive intestinal peptide (VIP) was first discovered, isolated and purified from porcine intestine. [US 3,879,371]. The peptide has twenty-eight (28) amino acids and bears extensive homology to secretin and glucagon. [Carlquist et al., Horm. Metab. Res., 14,28-29 (1982)]. The amino acid sequence of VIP is as follows:

His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn

(SEQ ID NO: 1)

VIP is known to exhibit a wide range of biological activities throughout the gastrointestinal tract and circulatory system. In light of its similarity to gastrointestinal hormones, VIP has been found to stimulate pancreatic and biliary secretion, hepatic glycogenolysis, glucagon and insulin secretion and to activate pancreatic bicarbonate release.

Two types of VIP receptors are known and have been cloned from human, rat, mouse, chicken, fish and frog. They are currently identified as VPAC1 and VPAC2 and respond to native VIP with comparable affinity. VPAC2 receptor mRNA is found in the human respiratory tract including tracheal and bronchial epithelium, glandular and immune cells, alveolar walls and macrophages. [Groneberg et al., Lab. Invest. 81:749-755 (2001) and Laburthe et al., Receptors and Channels 8:137-153 (2002)].

Neurons containing VIP have been localized by immunoassay in cells of the endocrine and exocrine systems, intestine and smooth muscle. VIP has been found to be a neuroeffector causing the release of several hormones including prolactin, thyroxine, and insulin and glucagon. VIP has also been found to stimulate renin release from the kidney in vivo and in vitro. VIP has been found to be present in nerves and nerve terminals in the airways of various animal species and man. VIP's cardiovascular and bronchopulmonary effects are of interest as VIP has been found to be a powerful vasodilator and potent smooth muscle relaxant, acting on peripheral, pulmonary, and coronary vascular beds. VIP has been found to have a vasodilatory effect on cerebral blood vessels. In vitro studies have demonstrated that vasoactive intestinal peptide, applied exogenously to cerebral arteries, induced vaso-

dilation, suggesting VIP as a possible transmitter for cerebral vasodilation. In the eye, VIP has also been shown to be a potent vasodilator.

VIP may have regulatory effects on the immune system, e.g. VIP can modulate the proliferation and migration of lymphocytes. Native VIP has been shown to inhibit IL-12 production in LPS-stimulated macrophages with effects on IFNγ synthesis. VIP inhibits TGF-β1 production in murine macrophages and inhibits IL-8 production in human monocytes through NFκB. [Sun et al., J. Neuroimmunol. 107:88-99 (2000) and Delgado and Ganea, Biochem. Biophys. Res. Commun. 302:275-283 (2003)].

Since VIP has been found to relax smooth muscle and it is normally present in airway tissues, as noted above, it has been hypothesized that VIP may be an endogenous mediator of bronchial smooth muscle relaxation. It has been shown that tissues from asthmatic patients contain no immunoreactive VIP, as compared to tissue from normal patients. This may be indicative of a loss of VIP or VIPergic nerve fibers associated with the disease of asthma. In vitro and in vivo testing have shown VIP to relax tracheal smooth muscle and protect against bronchoconstrictor agents such as histamine and prostaglandin $F_{2\alpha}$. When giving intravenously, VIP has been found to protect against bronchoconstrictor agents such as histamine, prostaglandin $F_{2\alpha}$, leukotrienes, platelet activating factor as well as antigen-induced bronchoconstrictions. VIP has also been found to inhibit mucus secretion in human airway tissue in vitro.

Disorders of the airways have diverse causes but share various pathophysiologic and clinical features. Characteristic of these disorders are limitation of airflow resulting from airway obstruction, thickening of airway walls, inflammation or loss of elasticity of interstitial tissue. Co-morbidities may include hypersecretion of mucus, airway hyperreactivity, and gas exchange abnormalities which may result on cough, sputum production, wheezing and dyspnea. Common disorders of the airways include: asthma, chronic obstructive pulmonary disease (COPD), chronic bronchitis, emphysema, and pulmonary hypertension. [Mayer et al., Respiration Physiol. 128:3-11 (2001)].

COPD is a group of chronic conditions defined by the obstruction of the lung airways. COPD includes two major breathing diseases which are chronic (obstructive) bronchitis and emphysema. Both diseases are associated with breathing difficulty and breathlessness. COPD may be accompanied by pulmonary hypertension. Long-term cigarette smoking is the predominant risk factor for COPD. The airway limitation associated with COPD is generally regarded as being irreversible.

Chronic bronchitis is a progressive inflammatory disease. Associated with this disease is an increase in mucus production in the airways and increase in the occurrence of bacterial infections. This chronic inflammatory condition induces thickening of the walls of the bronchi resulting in increased congestion and dyspnea.

Emphysema is an underlying pathology of COPD by damaging lung tissue with enlargement of the airspaces and loss of alveolar surface area. Lung damage is caused by weakening and breaking the air sacs within the lungs. Natural elasticity of the lung tissue is also lost, leading to overstretching and rupture. Smaller bronchial tubes may be damaged which can cause them to collapse and obstruct airflow, leading to shortage of breath.

COPD, in its substantial medical meaning, is always accompanied by bronchial obstruction. Thus, the most common symptoms of COPD include shortness of breath, chronic coughing, chest tightness, greater effort to breathe, increased mucus production and frequent clearing of the throat. Patients are unable to perform their usual daily activities. Independent development of chronic bronchitis and emphysema is possible, but most people with COPD have a combination of the disorders.

Breakdown of connective tissue in lung parenchyma, in particular elastin, results in the loss of elasticity found in many airway disorders. Evidence for elastin degradation has been shown in emphysema and COPD. Neutrophil elastase is considered to be a primary protease responsible for elastin destruction. [Barnes et al., Eur. Respir. J. 22:672-688 (2003)]. Production of neutrophil elastase has been shown to be enhanced in the lungs of COPD patients. [Higashimoto et al., Respiration 72:629-635 (2005)].

Because of the interesting and potential clinically useful biological activities of VIP, the peptide has been the target of several reported synthetic programs with the goal of enhancing one or more of the properties of this molecule. Takeyama et al. have reported a VIP analog having a glutamic acid substituted for aspartic acid at position 8. This compound was found to be less potent than native VIP. [Chem. Pharm. Bull. 28:2265-2269 (1980)]. Wendlberger et al. have disclosed the preparation of a VIP analog having a norleucine substituted at position 17 for methionine. [Peptide Proc. 16th Eur. Pept. Symp., 290-295 (1980)]. The peptide was found to be equipotent to native VIP for its ability to displace radioiodinated VIP from liver membrane preparations. Watts and Wooton have reported a series of linear and cyclic VIP fragments, containing between six and twelve residues from the native sequence. [EP 184,309, EP 325,044; US 4,737,487, US 4,866,039]. Turner et al have reported that the fragment VIP(10-28) is an antagonist to VIP [Peptides 7:849-854 (1986)]. The substituted analog [4-Cl-D-Phe^{6},Leu^{17}]-VIP has also been reported to bind to

the VIP receptor and antagonize the activity of VIP [Pandol et al., Gastrointest. Liver Physiol. 13:G553-G557 (1986)]. Gozes et al. have reported that the analog [Lys^1,Pro^2,Arg^3, Arg^4,Pro^5,Tyr^6]-VIP is a competitive inhibitor of VIP binding to its receptor on glial cells. [Endocrinology 125:2945-2949 (1989)]. Robberecht et al. have reported several VIP analogs with D-residues substituted in the N-terminus of native VIP. [Peptides 9:339-345 (1988)]. All of these analogs bound less tightly to the VIP receptor and showed lower activity than native VIP in c-AMP activation. Tachibana and Ito have reported several VIP analogs of the precursor molecule. [in: Peptide Chem. Shiba and Sakakibara (eds.), Prot. Res. Foundation, 1988, 481-486, JP 1083012, US 4,822,774]. These compounds were shown to be 1-to 3-fold more potent bronchodilators than VIP and had a 1-to 2-fold higher level of hypotensive activity. Musso et al. have also reported several VIP analogs have substitutions at positions 6-7, 9-13, 15-17, and 19-28. [Biochem 27:8174-8181 (1988); US 4,835,252]. These compounds were found to be equal to or less potent than native VIP in binding to the VIP receptor and in biological response. Bartfai et al have reported a series of multiply substituted [Leu^{17}]-VIP analogs. [WO 89/05857].

Gourlet et al have reported an [Arg^{16}]-VIP derivative with affinity for VIP receptors [BBA 1314:267-273 (1996)]. Onoue et al have reported a series of arginine derivatives and truncations of VIP [Onoue et al., Life Sci. 74:1465-77 (2004) and Ohmori et al., Regul. Pept. 123:201-207 (2004)]. A series of poly-alanine derivatives has also been reported [Igarashi et al., J. Pharm. Exper. Ther. 303:445-460 (2002) and Igarashi et al., J. Pharm. Exper. Ther. 315:370-81 (2005)].

In US20050203009 analogs of VIP having selective VPAC1 agonist activity are described. Analogs of VIP and C-terminal pegylated derivatives have been reported has being of utility for the treatment of metabolic disorders including diabetes [e.g. WO2006042152]. Peptides having VPAC2 agonist activity have been identified, and include PACAP and VIP analogs [Gourlet et al., Peptides 18:403-408; Xia et al., J. Pharmacol. Exp. Ther. 281:629-633 (1997)]. Cyclic analogs of VIP have been reported that have enhanced stability and activity [Bolin et al., Biopolymers 37:57-66 (1995), US 5,677,419].

In man, when administered by intravenous infusion to asthmatic patients. VIP has been shown to cause an increase in peak expiratory flow rate and protect against histamine-induced bronchodilation. [Morice and Sever, Peptides 7:279-280 (1986); Morice et al., The Lancet, II 1225-1227 (1983)]. The pulmonary effects observed by this intravenous infusion of VIP were, however, accompanied by cardiovascular side-effects, most notably hypotension and tachycardia and also facial flushing. When given in intravenous doses which did not cause cardiovascular effects, VIP failed to alter specific airway conductance. [Palmer et

al., Thorax 41:663-666 (1986)]. The lack of activity was explained as being due to the low dose administered and possibly due to rapid degradation of the compound. When administered by aerosol to humans, native VIP has been only marginally effective in protecting against histamine-induced bronchoconstriction. [Altieri et al., Pharmacologist 25:123 (1983)]. VIP was found to have no significant effect on baseline airway parameters but did have a protective effect against histamine-induced bronchoconstriction when given by inhalation to humans. [Barnes and Dixon, Am. Rev. Respir. Dis., 130:162-166 (1984)]. VIP, when given by aerosol, has been reported to display no tachycardia or hypotensive effects in conjunction with the bronchodilation. [Said et al., in: Vasoactive Intestinal Peptide, Said]ed.), Raven Press, New York, 1928, 185-191].

A derivative of VIP, RO 25-1553, has been reported to have efficacy as a bronchodilatory both preclinically and clinically in mild asthmatics [Kallstrom and Waldeck, Eur. J. Pharm. 430:335-40 (2001) and Linden et al., Thorax 58:217-21 (2003)]. Native VIP has been reported to be of utility for the treatment of COPD, pulmonary hypertension and other airway disorders [WO03061680, WO0243746 and WO2005014030].

A need exists, however, for novel analogs of vasoactive intestinal peptide having selectivity for the VPAC2 receptor, while possessing equal or better potency, pharmacokinetic properties and pharmacological properties than existing VPAC agonists. Preferably, a need exists for compounds having greater duration of activity than those previously available.

The present invention comprises a VPAC-2 receptor agonist of the formula (I):

X-His-R^2-Asp-Ala-R^5-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-R^{16}-Nle-R^{18}-Ala-Lys-$\underline{Lys^{21}}$-Tyr-Leu-Asn-$\underline{Asp^{25}}$-Leu-R^{27}-R^{28}-Gly-Gly-Thr-Y

[X-(SEQ ID NO: 2)-Y]

wherein

X is a hydrogen of the N-terminal amino of Histidine which may be optionally replaced by a hydrolyzable amino protecting group, most preferably by an acetyl group,

Y is the hydroxy of the C-terminal carboxy of Threonine which may be optionally replaced by a hydrolyzable carboxy protecting group, most preferably by NH_2,

underlined residues indicates a side-chain to side-chain covalent linkage of the first (Lys^{21}) and last (Asp^{25})amino acids within the segment,

R^2 is Ser or Ala,

R^5 is Thr, Ser, Asp, Gln, Pro or CαMeVal,

R^{16} is Gln, Ala, or Arg,

R^{18} is Ala, Lys or Glu,

R^{27} is Lys or Leu except that R^{27} must be Lys when R^5 is CαMeVal and R^{16} is Arg,

R^{28} is Lys or Asn,

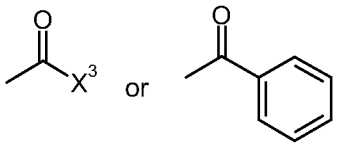
or a pharmaceutically acceptable salt thereof.

The compounds of the invention are active agonists of the VPAC2 receptor and have enhanced stability to human neutrophil elastase. Thus, the compounds, as selective stable analogs of native VIP having improved resistance to the effects of elastase present in the human lung, would be useful for the treatment of airway disorders, including COPD.

All peptide sequences mentioned herein are written according to the usual convention whereby the N-terminal amino acid is on the left and the C-terminal amino acid is on the right, unless noted otherwise. A short line between two amino acid residues indicates a peptide bond. A segment of amino acids with underline indicates a side-chain to side-chain covalent linkage of the first and last amino acids within the segment. Typically this is an amide bond. Where the amino acid has isomeric forms, it is the L form of the amino acid that is represented unless otherwise expressly indicated. For convenience in describing this invention, the conventional and nonconventional abbreviations for the various amino acids are used. These abbreviations are familiar to those skilled in the art, but for clarity are listed below:

Asp=D=Aspartic Acid; Ala=A=Alanine; Arg=R=Arginine; Asn=N=Asparagine; Gly=G=Glycine; Glu=E=Glutamic Acid; Gln=Q=Glutamine; His=H=Histidine; Ile=I=Isoleucine; Leu=L=Leucine; Lys=K=Lysine; Met=M=Methionine; MeVal=MeV= CαMeVal; Nle=Norleucine; Phe=F=Phenylalanine; Pro=P=Proline; Ser=S=Serine; Thr=T=Threonine; Trp=W=Tryptophan; Tyr=Y=Tyrosine; and Val=V=Valine.

With respect to the terms "hydrolyzable amino protecting group" and "hydrolyzable carboxy protecting group", any conventional protecting groups which can be removed by hydrolysis can be utilized in accordance with this invention. Examples of such groups appear hereinafter. Preferred amino protecting groups are acyl groups of the formula



wherein X^3 is lower alkyl or halo lower alkyl. Of these protecting groups, those wherein X^3 is C_1-C_3alkyl or halo-C_1-C_3alkyl are especially preferred. Preferred carboxy protecting

groups are lower alkyl esters, NH_2 and lower alkyl amides, with C_1-C_3alkyl esters, NH_2 and C_1-C_3alkyl amides being especially preferred.

Also for convenience, and readily known to one skilled in the art, the following abbreviations or symbols are used to represent the moieties, reagents and the like used in this invention: Nle: norleucine; CαMeVal: Cα-methyl-L-valine; MeVal: Cα-methyl-L-valine; CH_2Cl_2: methylene chloride; Ac: acetyl; Ac_2O: acetic anhydride; AcOH: acetic acid; ACN: acetonitrile; DMAc: dimethylacetamide; DMF: dimethylformamide; DIPEA: N, N-diisopropylethylamine; TFA: trifluoroacetic acid; HOBT: N-hydroxybenzotriazole; DIC: N, N'-diisopropylcarbodiimide; BOP: benzotriazol-1-yloxy-tris- (dimethylamino) phosphonium-hexafluorophosphate; HBTU: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphate; NMP: 1-methyl-2-pyrrolidinone; MALDI-TOF: matrix assisted laser desorption ionization-time of flight; FAB-MS: fast atom bombardment mass spectrometry; ES-MS: electro spray mass spectrometry; RT: room temperature.

As used herein, the term "alkyl" means a branched or unbranched, cyclic or acyclic, saturated or unsaturated (e.g. alkenyl or alkynyl) hydrocarbyl radical which may be substituted or unsubstituted. Where cyclic, the alkyl group is preferably C_3 to C_{12}, more preferably C_5 to C_{10}, more preferably C_5 to C_7. Where acyclic, the alkyl group is preferably C_1 to C_{10}, more preferably C_1 to C_6, more preferably methyl, ethyl, propyl (n-propyl or isopropyl), butyl (n-butyl, isobutyl or tertiary-butyl) or pentyl (including n-pentyl and isopentyl), more preferably methyl.

As used herein, the term "lower alkyl" means a branched or unbranched, cyclic or acyclic, saturated or unsaturated (e.g. alkenyl or alkynyl) hydrocarbyl radical wherein said cyclic lower alkyl group is C_5, C_6 or C_7, and wherein said acyclic lower alkyl group is C_1, C_2, C_3 or C_4, and is preferably selected from methyl, ethyl, propyl (n-propyl or isopropyl) or butyl (n-butyl, isobutyl or tertiary-butyl).

As used herein, the term "acyl" means an optionally substituted alkyl, cycloalkyl, heterocyclic, aryl or heteroaryl group bound via a carbonyl group and includes groups such as acetyl, propionyl, benzoyl, 3-pyridinylcarbonyl, 2-morpholinocarbonyl, 4-hydroxybutanoyl, 4-fluorobenzoyl, 2-naphthoyl, 2-phenylacetyl, 2-methoxyacetyl and the like.

As used herein, the term "aryl" means a substituted or unsubstituted carbocyclic aromatic group, such as phenyl or naphthyl, or a substituted or unsubstituted heteroaromatic group containing one or more, preferably one, heteroatom.

The alkyl and aryl groups may be substituted or unsubstituted. Where substituted, there will generally be 1 to 3 substituents present, preferably 1 substituent. Substituents may include: carbon-containing groups such as alkyl, aryl, arylalkyl (e.g. substituted and unsubstituted phenyl, substituted and unsubstituted benzyl); halogen atoms and halogen-containing groups such as haloalkyl (e.g. trifluoromethyl); oxygen-containing groups such as alcohols (e.g. hydroxyl, hydroxyalkyl, aryl(hydroxyl)alkyl), ethers (e.g. alkoxy, aryloxy, alkoxyalkyl, aryloxyalkyl), aldehydes (e.g. carboxaldehyde), ketones(e.g. alkylcarbonyl, alkylcarbonylalkyl, arylcarbonyl, arylalkylcarbonyl, arycarbonylalkyl), acids (e.g. carboxy, carboxyalkyl), acid derivatives such as esters(e.g. alkoxycarbonyl, alkoxycarbonylalkyl, alkylcarbonyloxy, alkylcarbonyloxyalkyl), amides (e.g. aminocarbonyl, mono- or di-alkylaminocarbonyl, aminocarbonylalkyl, mono-or di-alkylaminocarbonylalkyl, arylaminocarbonyl), carbamates (e.g. alkoxycarbonylamino, arloxycarbonylamino, aminocarbonyloxy, mono- or di-alkylaminocarbonyloxy, arylminocarbonloxy) and ureas (e.g. mono- or di-alkylaminocarbonylamino or arylaminocarbonylamino); nitrogen-containing groups such as amines (e.g. amino, mono- or di-alkylamino, aminoalkyl, mono- or di-alkylaminoalkyl), azides, nitriles (e.g. cyano, cyanoalkyl), nitro; sulfur-containing groups such asthiols, thioethers, sulfoxides and sulfones (e.g. alkylthio, alkylsulfinyl, alkylsulfonyl, alkylthioalkyl, alkylsulfinylalkyl, alkylsulfonylalkyl, arylthio, arysulfinyl, arysulfonyl, arythioalkyl, arylsulfinylalkyl, arylsulfonylalkyl); and heterocyclic groups containing one or more, preferably one, heteroatom.

As used herein, the term “halogen” means a fluorine, chlorine, bromine or iodine radical, preferably a fluorine, chlorine or bromine radical, and more preferably a fluorine or chlorine radical.

“Pharmaceutically acceptable salt” refers to conventional acid-addition salts or base-addition salts that retain the biological effectiveness and properties of the compounds of formula I and are formed from suitable non-toxic organic or inorganic acids or organic or inorganic bases. Sample acid-addition salts include those derived from inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, sulfamic acid, phosphoric acid and nitric acid, and those derived from organic acids such as p-toluenesulfonic acid, salicylic acid, methanesulfonic acid, oxalic acid, succinic acid, citric acid, malic acid, lactic acid, fumaric acid, and the like. Sample base-addition salts include those derived from ammonium, potassium, sodium and, quaternary ammonium hydroxides, such as e.g., tetramethylammonium hydroxide. The chemical modification of a pharmaceutical compound (i.e. drug) into a salt is a well known technique which is used in attempting to improve properties involving physical or chemical stability, e.g., hygroscopicity, flowability

or solubility of compounds. *See, e.g.,* Ansel et. al., Pharmaceutical Dosage Forms and Drug Delivery Systems (6th Ed. 1995) at pp. 196 and 1456-1457.

"Pharmaceutically acceptable ester" refers to a conventionally esterified compound of formula I having a carboxyl group, which esters retain the biological effectiveness and properties of the compounds of formula I and are cleaved *in vivo* (in the organism) to the corresponding active carboxylic acid. Examples of ester groups which are cleaved (in this case hydrolyzed) *in vivo* to the corresponding carboxylic acids are those in which the cleaved hydrogen is replaced with -lower alkyl which is optionally substituted, e.g., with heterocycle, cycloalkyl, etc. Examples of substituted lower alkyl esters are those in which lower alkyl is substituted with pyrrolidine, piperidine, morpholine, N-methylpiperazine, etc. The group which is cleaved *in vivo* may be, e.g., ethyl, morpholino ethyl, and diethylamino ethyl. In connection with the present invention, $-CONH_2$ is also considered an ester, as the $-NH_2$ is cleaved *in vivo* and replaced with a hydroxy group, to form the corresponding carboxylic acid.

Further information concerning examples of and the use of esters for the delivery of pharmaceutical compounds is available in Design of Prodrugs, Bundgaard (ed.) (Elsevier, 1985). *See also,* Ansel et. al., Pharmaceutical Dosage Forms and Drug Delivery Systems (6th Ed. 1995) at pp. 108-109; Krogsgaard-Larsen et. al., Textbook of Drug Design and Development (2d Ed. 1996) at pp. 152-191.

In one embodiment the present invention provides a compound of formula I wherein X is a hydrogen of the N-terminal amino of Histidine or wherein said hydrogen is replaced by an acetyl group. In another embodiment the present invention provides a compound of formula I wherein X is a hydrogen of the N-terminal amino of Histidine.

In one embodiment the present invention provides a compound of formula I wherein Y is the hydroxy of the C-terminal carboxy of Threonine or wherein said hydroxy is replaced by NH_2. In another embodiment the present invention provides a compound of formula I wherein Y is the hydroxy of the C-terminal carboxy of Threonine.

In one embodiment the present invention provides a compound of formula I wherein R^2 is Ser. In another embodiment the present invention provides a compound of formula I wherein R^2 is Ala.

In one embodiment the present invention provides a compound of formula I wherein R^5 is Thr, Ser or CαMeVal. In another embodiment the present invention provides a compound of formula I wherein R^5 is Thr. In another embodiment the present invention provides a

compound of formula I wherein R^5 is Ser. In another embodiment the present invention provides a compound of formula I wherein R^5 is CαMeVal.

In one embodiment the present invention provides a compound of formula I wherein R^{16} is Gln or Arg. In another embodiment the present invention provides a compound of formula I wherein R^{16} is Gln. In another embodiment the present invention provides a compound of formula I wherein R^{16} is Arg.

In one embodiment the present invention provides a compound of formula I wherein R^{18} is Ala. In another embodiment the present invention provides a compound of formula I wherein R^{18} is Lys. In another embodiment the present invention provides a compound of formula I wherein R^{18} is Glu.

In one embodiment the present invention provides a compound of formula I wherein R^{27} is Lys.

In one embodiment the present invention provides a compound of formula I wherein R^{28} is Lys.

In one embodiment the present invention provides a compound of formula I wherein

X is a hydrogen of the N-terminal amino of Histidine or said hydrogen replaced by an acetyl group,

Y is the hydroxy of the C-terminal carboxy of Threonine or said hydroxy replaced by NH_2,

R^2 is Ser or Ala,

R^5 is Thr, Ser or CαMeVal,

R^{16} is Gln or Arg,

R^{18} is Ala, Lys or Glu,

R^{27} is Lys or Leu except that R^{27} must be Lys when R^5 is CαMeVal and R^{16} is Arg, and

R^{28} is Lys.

In another embodiment the present invention provides a compound of formula I wherein

X is a hydrogen of the N-terminal amino of Histidine or said hydrogen replaced by an acetyl group,

Y is the hydroxy of the C-terminal carboxy of Threonine or said hydroxy replaced by NH_2,

R^2 is Ser or Ala,

R^5 is Thr, Ser or CαMeVal,

R^{16} is Gln or Arg,

R^{18} is Ala, Lys or Glu,

R^{27} is Lys or Leu except that R^{27} must be Lys when R^5 is CαMeVal and R^{16} is Arg, and

R^{28} is Lys.

In another embodiment the present invention provides a compound of formula I wherein

X is a hydrogen of the N-terminal amino of Histidine or said hydrogen replaced by an acetyl group,

Y is the hydroxy of the C-terminal carboxy of Threonine or said hydroxy replaced by NH_2,

R^2 is Ser or Ala,

R^5 is Ser or CαMeVal,

R^{16} is Gln,

R^{18} is Ala,

R^{27} is Lys or Leu, and

R^{28} is Lys.

The present representative compounds may be readily synthesized by any known conventional procedure for the formation of a peptide linkage between amino acids. Such conventional procedures include, e.g., any solution phase procedure permitting a condensation between the free alpha amino group of an amino acid or residue thereof having its carboxyl group and other reactive groups protected and the free primary carboxyl group of another amino acid or residue thereof having its amino group or other reactive groups protected.

Such conventional procedures for synthesizing the novel compounds of the present invention include e.g. any solid phase peptide synthesis method. In such a method the synthesis of the novel compounds can be carried out by sequentially incorporating the desired amino acid residues one at a time into the growing peptide chain according to the general principles of solid phase methods. Such methods are disclosed in, e.g., Merrifield, J. Amer. Chem. Soc. 85:2149-2154 (1963); Barany et al., The Peptides, Analysis, Synthesis and Biology, Vol. 2, Gross and Meienhofer, (Eds.) Academic Press 1-284 (1980), which are incorporated herein by reference. Peptide synthesis may be performed manually or with automated instrumentation. Microwave-assisted synthesis may also be utilized.

Common to chemical syntheses of peptides is the protection of reactive side chain groups of the various amino acid moieties with suitable protecting groups, which will prevent a chemical reaction from occurring at that site until the protecting group is ultimately re-

moved. Usually also common is the protection of the alpha amino group on an amino acid or fragment while that entity reacts at the carboxyl group, followed by the selective removal of the alpha amino protecting group at allow a subsequent reaction to take place at that site. While specific protecting groups have been disclosed in regard to the solid phase synthesis method, it should be noted that each amino acid can be protected by a protective group conventionally used for the respective amino acid in solution phase synthesis.

Alpha amino groups may be protected by a suitable protecting group selected from aromatic urethane-type protecting groups, such as allyloxycarbonyl, benzyloxycarbonyl (Z) and substituted benzyloxycarbonyl, such as p-chlorobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, p-biphenyl-isopropyloxycarbonyl, 9-fluorenylmethyloxycarbonyl (Fmoc) and p-methoxybenzyloxycarbonyl (Moz); aliphatic urethane-type protecting groups, such as t-butyloxycarbonyl (Boc), diisopropylmethyloxycarbonyl, isopropyloxycarbonyl, and allyloxycarbonyl. Herein, Fmoc is most preferred for alpha amino protection.

Guanidino groups may be protected by a suitable protecting group selected from nitro, p-toluenesulfonyl (Tos), (Z,) 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc); 4-methoxy-2,3,6,-trimethylbenzenesulfonyl (Mtr). Pmc and Mtr are most preferred for arginine (Arg).

The ε-amino groups may be protected by a suitable protecting group selected from 2-chloro-benzyloxycarbonyl (2-Cl-Z), 2-bromo-benzyloxycarbonyl (2-Br-Z) - and Boc. Boc is the most preferred for (Lys).

Hydroxyl groups (OH) may be protected by a suitable protecting group selected from benzyl (Bzl), 2, 6 dichlorobenzyl (2,6-diCl-Bzl), and tert-butyl (t-Bu). tBu is most preferred for (Tyr), (Ser) and (Thr).

The β- and γ-amide groups may be protected by a suitable protecting group selected from 4-methyltrityl (Mtt), 2, 4, 6-trimethoxybenzyl (Tmob), 4, 4-dimethoxydityl/bis-(4-methoxyphenyl)-methyl (Dod) and trityl (Trt). Trt is the most preferred for (Asn) and (Gln).

The indole group may be protected by a suitable protecting group selected from formyl (For), mesityl-2-sulfonyl (Mts) and Boc. Boc is the most preferred for (Trp).

The β- and γ-carboxyl groups may be protected by a suitable protecting group selected from t-butyl (tBu), and 2-phenylisopropyl ester (2Pip). tBu is the most preferred for (Glu) and 2Pip is most preferred for (Asp).

The imidazole group may be protected by a suitable protecting group selected from benzyl (Bzl), Boc, and trityl (Trt). Trt is the most preferred for (His).

All solvents, isopropanol (iPrOH), methylene chloride (CH_2Cl_2), DMF and NMP were purchased from Fisher, JT Baker or Burdick & Jackson and were used without additional distillation. TFA was purchased from Halocarbon, Aldrich or Fluka and used without further purification.

DIC and DIPEA was purchased from Fluka or Aldrich and used without further purification. HOBT, dimethylsulfide (DMS) and 1, 2-ethanedithiol (EDT) were purchased from Aldrich, Sigma Chemical Co. or Anaspec and used without further purification. Protected amino acids were generally of the L configuration and were obtained commercially from Bachem, Advanced ChemTech, CEM or Neosystem. Purity of these reagents was confirmed by thin layer chromatography, NMR and melting point prior to use. Benzhydrylamine resin (BHA) was a copolymer of styrene - 1% divinylbenzene (100-200 or 200-400 mesh) obtained from Bachem, Anaspec or Advanced Chemtech. Total nitrogen content of these resins were generally between 0.3 - 1.2 meq/g.

High performance liquid chromatography (HPLC) was conducted on a LDC apparatus consisting of Constametric I and III pumps, a Gradient Master solvent programmer and mixer, and a Spectromonitor III variable wavelength UV detector. Analytical HPLC was performed in reversed phase mode using Pursuit C_{18} columns (4.5 x 50 mm). Preparative HPLC separations were run on Pursuit columns (50 x 250 mm).

In a preferred embodiment, peptides were prepared using solid phase synthesis by the method generally described by Merrifield (J. Amer. Chem. Soc. 85:2149 (1963)), although other equivalent chemical synthesis known in the art could be used as previously mentioned. Solid phase synthesis is commenced from the C-terminal end of the peptide by coupling a protected alpha-amino acid to a suitable resin. Such a starting material can be prepared by attaching an alpha-amino-protected amino acid by an ester linkage to a p-benzyloxybenzyl alcohol (Wang) resin, or by an amide bond between an Fmoc-Linker, such as p-((R, S)-α-(1-(9H-fluoren-9-yl)-methoxyformamido)-2,4-dimethyloxybenzyl)-phenoxyacetic acid (Rink linker) to a benzhydrylamine (BHA) resin. Preparation of the hydroxymethyl resin is well known in the art. Fmoc-Linker-BHA resin supports are commercially available and generally used when the desired peptide being synthesized has an unsubstituted amide at the C-terminus.

Typically, the amino acids or mimetic are coupled onto the Fmoc-Linker-BHA resin using the Fmoc protected form of amino acid or mimetic, with 1 - 5 equivalents of amino acid and a suitable coupling reagent. After couplings, the resin may be washed and dried under vacuum. Loading of the amino acid onto the resin may be determined by amino acid analysis of an aliquot of Fmoc-amino acid resin or by determination of Fmoc groups by UV analysis. Any unreacted amino groups may be capped by treating the resin with acetic anhydride and diispropylethylamine in methylene chloride or DMF.

The resins are carried through several repetitive cycles to add amino acids sequentially. The alpha amino Fmoc protecting groups are removed under basic conditions. Piperidine, piperazine or morpholine (20-40% v/v) in DMF may be used for this purpose. Preferably 40% piperidine in DMF is typically utilized

Following the removal of the alpha amino protecting group, the subsequent protected amino acids are coupled stepwise in the desired order to obtain an intermediate, protected peptide-resin. The activating reagents used for coupling of the amino acids in the solid phase synthesis of the peptides are well known in the art. For example, appropriate reagents for such syntheses are BOP, Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP), HBTU and DIC. Preferred here are HBTU and DIC. Other activating agents are described by Barany and Merrifield (in: The Peptides, Vol. 2, Meienhofer (ed.), Academic Press, 1979, pp 1-284) may be utilized. Various reagents such as HOBT, N-hydroxysuccinimide (HOSu) and 3, 4-dihydro-3-hydroxy-4-oxo-1, 2, 3-benzotriazine (HOOBT) may be added to the coupling mixtures in order to optimize the synthetic cycles. Preferred here is HOBT.

The protocol for a typical synthetic cycle is as follows:

Protocol 1	Step	Reagent	Time
	1	DMF	2 x 30 sec
	2	20% piperidine/DMF	1 min
	3	20% piperidine/DMF	15 min
	4	DMF	2 x 30 sec
	5	iPrOH	2 x 30 sec
	6	DMF	3 x 30 sec
	7	Coupling	60 min - 18 hours
	8	DMF	2 x 30 sec
	9	iPrOH	1 x 30 sec

	10	DMF	1 x 30 sec
	11	CH_2Cl_2	2 x 30 sec

Solvents for all washings and couplings were measured to volumes of 10 - 20 ml/g resins. Coupling reactions throughout the synthesis were monitored by the Kaiser ninhydrin test to determine extent of completion [Kaiser et al., Anal.Biochem. 34:595-598 (1970)]. Any incomplete coupling reactions were either recoupled with freshly prepared activated amino acid or capped by treating the peptide resin with acetic anhydride as described above. The fully assembled peptide-resins were dried in vacuum for several hours.

Peptide synthesis may be performed using an Applied Biosystem 433A synthesizer (Foster City, CA), The FastMoc 0.25 mmole cycles were used with either the resin sampling or non resin sampling, 41 mL reaction vessel. The Fmoc-amino acid resin was dissolved with 2.1 g NMP, 2g of 0.45M HOBT/HBTU in DMF and 2M DIEA, then transferred to the reaction vessel. The basic FastMoc coupling cycle was represented by the module "BADEIFD," wherein each letter represents a module. For example: B represents the module for Fmoc deprotection using 20% piperidine/NMP and related washes and readings for 30 min (either UV monitoring or conductivity); A represents the module for activation of amino acid in cartridges with 0.45 M HBTU/HOBt and 2.0 M DIEA and mixing with N2 bubbling; D represents the module for NMP washing of resin in the reaction vessel; E represents the module for transfer of the activated amino acid to the reaction vessel for coupling; I represents the module for a 10 min waiting period with vortexing on and off of the reaction vessel; and F represents the module for cleaning cartridge, coupling for approximately 10 min and draining the reaction vessel. Couplings were typically extended by addition of module "I" once or multiple times. For example, double couplings were run by performing the procedure "BADEIIADEIFD." Other modules were available such as c for methylene chloride washes and "C" for capping with acetic anhydride. Individual modules were also modifiable by, e.g., changing the timing of various functions, such as transfer time, in order to alter the amount of solvent or reagents transferred. The cycles above were typically used for coupling one amino acid. For synthesizing tetra peptides, however, the cycles were repeated and strung together. For example, BADEIIADEIFD was used to couple the first amino acid, followed by BADEIIADEIFD to couple the second amino acid, followed by BADEIIADEIFD to couple the third amino acid, followed by BADEIIADEIFD to couple the fourth amino acid, followed by BIDDcc for final deprotection and washing.

Peptide synthesis may be performed using a Microwave Peptide Synthesizer, Liberty (CEM Corporation, Matthews, NC). The synthesizer was programmed for double coupling and capping by modification of preloaded 0.25mmol cycle. The microwave editor was used to program microwave power methods for use during the Fmoc deprotection, amino acid coupling and capping with acetic anhydride. This type of microwave control allows for methods to be created that control a reaction at a set temperature for a set amount of time. The Liberty automatically regulates the amount of power delivered to the reaction to keep the temperature at the set point. The default cycles for amino acid addition and final de-protection were selected in cycle editor and were automatically loaded while creating a peptide.

The synthesis was carried out on a 0.25 mmol scale using Fmoc-Linker-BHA resin (450 mg, 0.25 mmol). Resin was added to the 30 mL reaction vessel with 10 mL of DMF. Fmoc deprotection was performed with a 20% piperidine in DMF solution. For each amino acid coupling, Fmoc protected amino acid was dissolved in DMF to make a 0.2M solution and was added to the reaction vessel. All coupling reactions were performed with 0.5M HOBT/-HBTU and 2M DIEA/NMP. Any incomplete coupling reactions were either recoupled with freshly prepared activated amino acid or capped by treating the peptide resin with 25% acetic anhydride in DMF. Each deprotection, coupling and capping reaction was done using Microwave at 70°C for 300 seconds at 50 watts power and nitrogen bubbling.

For each amino acid coupling following 0.25 mmol coupling cycle was used:

Protocol 2	Transfer resin to vessel Add Piperidine Deprotection (10 mL) Microwave method for deprotection (50 watts; 70°C; 300 seconds) Wash resin with DMF (10 mL) Add Amino acid (5mL) Add Activator (HOBT/HBTU) (2mL) Add Activator base (DIEA) (1mL) Microwave method for Coupling (50 watts; 70°C; 300 seconds) Wash resin with DMF (10 mL) Add Amino acid (5 mL) Add Activator (HOBT/HBTU) (2 mL) Add Activator base (DIEA) (1 mL) Microwave method for Coupling (50 watts; 70°C; 300 seconds) Wash resin with DMF (10 mL)

	Add capping (Acetic Anhydride 10 mL) Microwave Method (capping) (50 watts; 70°C; 300 seconds) Wash resin with DMF (10 mL)

For synthesis of compounds presented here, a preferred synthetic procedure is shown in Scheme 1.

Scheme 1



Treatment of Fmoc-Rink-MBHA resin, 1, with piperdine/DMF followed by coupling with Fmoc-AA(P)^{31} with a reagent such as DIC, BOP or HBTU, where AA31 represents the 31st

amino acid residue and P represents an appropriate protecting group, yields Fmoc-$AA(P)^{31}$-Rink-Resin, **2**. Repetition of steps 1 & 2 for 30 cycles by adding the appropriate protected amino acid at each cycle, yields peptide resin **3**. The side chain protecting groups on AA^{25} and AA^{21} are removed by treatment with 2% TFA in CH_2Cl_2 and $PdCl_2/nBu_3SnH$, respectively. The side chain amine and carboxyl of AA^{21} and AA^{25} are cyclized by treatment with BOP and NMM in DMF to yield **4**.

For each compound, the blocking groups are removed and the peptide cleaved from the resin in the same step. For example, the peptide-resins may be treated with 100 µL ethanedithiol, 100 µl dimethylsulfide, 300 µL anisole, and 9.5 mL TFA, per gram of resin, at RT for 180 min. Or alternately, the peptide-resins may be treated with 1.0 mL triisopropyl silane and 9.5 mL TFA, per gram of resin, at RT for 180 min. The resin is filtered off and the filtrates are precipitated in chilled ethyl ether. The precipitates are centrifuged and the ether layer is decanted. The residue is washed with two or three volumes of Et_2O and re-centrifuged. The crude product **5** is dried under vacuum.

Purifications of the crude peptides are performed on Shimadzu LC-8A system by high performance liquid chromatography (HPLC) on a reverse phase Pursuit C-18 Column (50x250 mm. 300 Å, 10 µm). The peptides are dissolved in a minimum amount of water and acetonitrile and are injected in a column. Gradient elution is generally started at 2% B buffer, 2% -70% B over 70 min, (buffer A: 0.1% TFA/H_2O, buffer B: 0.1% TFA/CH_3CN) at a flow rate of 50 ml/min. UV detection is made at 220/280 nm. The fractions containing the products are separated and their purity is judged on Shimadzu LC-10AT analytical system using reverse phase Pursuit C18 column (4.6 x 50mm) at a flow rate of 2.5 ml/min., gradient (2-70 %) over 10 min.[buffer A: 0.1% TFA/H_2O, buffer B: 0.1% TFA/CH_3CN)]. Fractions judged to be of sufficient purity are pooled and lyophilized.

Purity of the final products is checked by analytical HPLC on a reversed phase column as stated above. All final products are also subjected to fast atom bombardment mass spectrometry (FAB-MS) or electrospray mass spectrometry (ES-MS). In the Examples, all products yielded the expected parent M+H ions within acceptable limits.

Analogs of VIP described in the invention are agonists of the VPAC2 receptor as demonstrated in Example 25. According to the elastase stability experiments in Example 25, such compounds have enhanced stability to human neutrophil elastase. Therefore, administration of these VPAC2 receptor agonists would be of utility for the treatment of airway disorders such as COPD.

The compounds of the present invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those formed with pharmaceutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, trifluoroacetic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids, such as hydrohalic acids (e.g., hydrochloric acid), sulfuric acid, or phosphoric acid and the like. Any procedure for obtaining a pharmaceutically acceptable salt known to a skilled artisan can be used.

In the practice of the method of the present invention, an effective amount of any one of the peptides of this invention or a combination of any of the peptides of this invention or a pharmaceutically acceptable salt thereof, is administered via any of the usual and acceptable methods known in the art, either singly or in combination. The compounds or compositions can thus be administered orally (e.g., buccal cavity), sublingually, parenterally (e.g., intramuscularly, intravenously, or subcutaneously), rectally (e.g., by suppositories or washings), transdermally (e.g., skin electroporation) or by inhalation (e.g., by aerosol), and in the form of solid, liquid or gaseous dosages, including tablets and suspensions. The administration can be conducted in a single unit dosage form with continuous therapy or in a single dose therapy ad libitum. The therapeutic composition can also be in the form of an oil emulsion or dispersion in conjunction with a lipophilic salt such as pamoic acid, or in the form of a biodegradable sustained-release composition for subcutaneous or intramuscular administration.

Thus, the method of the present invention is practiced when relief of symptoms is specifically required or perhaps imminent. Alternatively, the method of the present invention is effectively practiced as continuous or prophylactic treatment.

Useful pharmaceutical carriers for the preparation of the compositions hereof, can be solids, liquids or gases; thus, the compositions can take the form of tablets, pills, capsules, suppositories, powders, enterically coated or other protected formulations (e.g. binding on ion-exchange resins or packaging in lipid-protein vesicles), sustained release formulations, solutions, suspensions, elixirs, aerosols, and the like. The carrier can be selected from the various oils including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, and the like. Water, saline, aqueous dextrose, and glycols are preferred liquid carriers, particularly (when isotonic with the blood) for injectable solutions. For example, formulations for intravenous administration comprise sterile aqueous solutions of the active ingredient(s) which are prepared by dissolving solid active ingredient(s) in water to produce an aqueous solution, and rendering the solution sterile.

Suitable pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, gelatin, malt, rice, flour, chalk, silica, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The compositions may be subjected to conventional pharmaceutical additives such as preservatives, stabilizing agents, wetting or emulsifying agents, salts for adjusting osmotic pressure, buffers and the like. Suitable pharmaceutical carriers and their formulation are described in Remington's Pharmaceutical Sciences by E. W. Martin. Such compositions will, in any event, contain an effective amount of the active compound together with a suitable carrier so as to prepare the proper dosage form for proper administration to the recipient.

The dose of a compound of the present invention depends on a number of factors, such as, e.g., the manner of administration, the age and the body weight of the subject, and the condition of the subject to be treated, and ultimately will be decided by the attending physician or veterinarian. Such an amount of the active compound as determined by the attending physician or veterinarian is referred to herein, and in the claims, as an "effective amount". For example, the dose for inhalation administration is typically in the range of about 0.5 to about 100 μg/kg body weight. Preferably, the compound of the present invention is administered at a dose rate of from about 1μg/kg to about 50 μg/kg/day.

Representative delivery regimens include oral, parenteral (including subcutaneous, intramuscular and intravenous), rectal, buccal (including sublingual), transdermal, pulmonary and intranasal. The preferred route of administration is pulmonary administration by oral inhalation. Methods of pulmonary administration may include aerosolization of an aqueous solution of the cyclic peptides of the present invention or the inspiration of micronized dry powder formulations. Aerosolized compositions may include the compound packaged in reverse micelles or liposomes. The preparation of micronized powders of suitably controlled particle size to effectively provide for alveolar delivery is well known. Inhalers for the delivery of specified doses of such formulations directly into the lungs (Metered Dose Inhalers or “MDIs”) are well known in the art.

Thus, the present invention also encompasses pharmaceutical compositions containing such agonists, and the use of such agonists for the treatment of pulmonary diseases including COPD.

In one embodiment the invention provides a pharmaceutical composition for inhalation administration comprising a compound of formula I and at least one pharmaceutically acceptable carrier or excipient in solution or micronized dry powder form wherein the

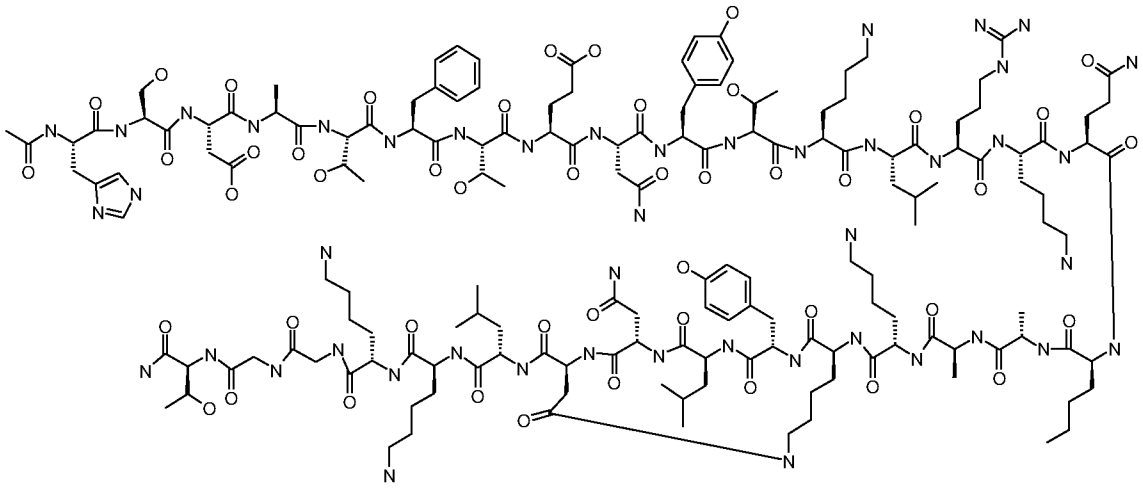
compound is present in a pharmacologically effective concentration for pulmonary delivery of said composition. In another embodiment the invention provides a pharmaceutical composition for inhalation administration comprising a compound of formula I and at least one pharmaceutically acceptable carrier or excipient in solution or micronized dry powder form wherein the concentration of the compound is sufficient to deliver from about 1 μg/kg to about 50 μg/kg of the compound in a single inhaled dose.

In one embodiment the invention provides a method for treating pulmonary obstructive disorders, e.g. COPD, comprising administering by inhalation an effective amount, e.g. from about 1 μg/kg/day to about 50 μg/kg/day, of a pharmaceutical composition for inhalation administration comprising a compound of formula I and at least one pharmaceutically acceptable carrier or excipient in solution or micronized dry powder form wherein the compound is present in a pharmacologically effective concentration for pulmonary delivery of said composition, e.g. to a person suffering from such disorder.

The invention will now be further described in the following Examples, which are intended as an illustration only and do not limit the scope of the invention.

EXAMPLES

Example 1: Preparation of Ac-His-Ser-Asp-Ala-Thr-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:3)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Thr, R^{16} is Gln, R^{18} is Ala, R^{27} is Lys and R^{28} is Lys



The above peptide was synthesized using Fmoc chemistry on an Applied Biosystem 433A or a microwave Peptide synthesizer. The synthesizer was programmed for double coupling using the modules described in Protocol 1 or 2 above. The synthesis was carried out on a 0.25 mmol scale using the Fmoc-Rink Linker-BHA resin (450 mg, 0.25 mmol). At the end of the synthesis, the resin was transferred to a reaction vessel on a shaker. The peptide resin in DMF was filtered and washed with CH_2Cl_2. The resin was treated five times with 2% TFA in CH_2Cl_2 for 3 min each. The resin was immediately treated twice with 5% DIPEA/CH_2Cl_2 and washed with CH_2Cl_2 and DMF. The peptide resin was suspended in DMF in a shaker vessel securely fitted with a rubber septum. To this was added 60 mg $PdCl_2(Ph_3P)_2$, 150 µl morpholine and 300 µl AcOH. The vessel was purged well with Ar. nBu_3SnH was then added via syringe. The black solution was shaken for 30-45 min, washed with DMF and repeated. Following the second Pd treatment, the resin was washed with DMF, 2 x iPrOH, DMF, 5% DIPEA/DMF and DMF. In DMF, the peptide resin was cyclized by treatment with BOP and NMM overnight. The resin was washed with DMF and CH_2Cl_2 and then dried under vacuum.

The peptide was cleaved from the resin using 13.5 mL 97% TFA/ 3%H_2O and 1.5mL tri-isopropylsilane for 180 min at RT. The deprotection solution was added to 100 mL cold Et_2O, and washed with 1 mL TFA and 30 mL cold Et_2O to precipitate the peptide. The peptide was centrifuged in two 50 mL polypropylene tubes. The precipitates from the indivi-

dual tubes were combined in a single tube and washed 3 times with cold Et_2O and dried in a desiccator under house vacuum.

The crude material was purified by preparative HPLC on a Pursuit C18-Column (250 x 50 mm, 10 µm particle size) and eluted with a linear gradient of 2-70%B (buffer A: 0.1 % TFA/H_2O; buffer B: 0.1% TFA/CH_3CN) in 90 min., flow rate 60mL/min,and detection 220/280 nm. The fractions were collected and were checked by analytical HPLC. Fractions containing pure product were combined and lyophilized to yield 106 mg (9.7%) of a white amorphous powder. (ES)+-LCMS m/e calculated ("calcd") for $C_{159}H_{256}N_{46}O_{47}$ 3565.05 found 3563.7.

Example 2: Preparation of Ac-His-Ser-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:4)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Ser, R^{16} is Gln, R^{18} is Ala, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 28 mg (2.5%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{158}H_{254}N_{46}O_{47}$ 3551.02 found 3548.7.

Example 3: Preparation of Ac-His-Ser-Asp-Ala-Asp-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:5)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Asp, R^{16} is Gln, R^{18} is Ala, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 9.2 mg (1%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{159}H_{254}N_{46}O_{48}$ 3579.03 found 3577.8.

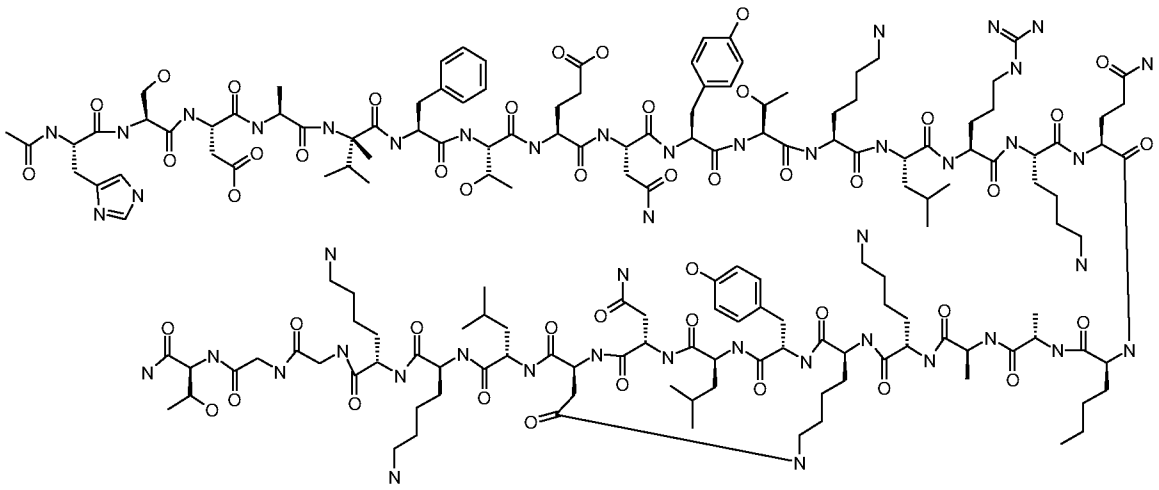
Example 4: Preparation of Ac-His-Ser-Asp-Ala-Gln-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:6)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Gln, R^{16} is Gln, R^{18} is Ala, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 9.8 mg (1%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{160}H_{257}N_{47}O_{47}$ 3592.07 found 3589.5.

Example 5: Preparation of Ac-His-Ser-Asp-Ala-Pro-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:7)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Pro, R^{16} is Gln, R^{18} is Ala, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 15.2 mg (1.4%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{160}H_{256}N_{46}O_{46}$ 3561.06 found 3560.0.

Example 6: Preparation of Ac-His-Ser-Asp-Ala-MeVal-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:8)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is MeVal, R^{16} is Gln, R^{18} is Ala, R^{27} is Lys and R^{28} is Lys



Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 40 mg (3.6%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{161}H_{260}N_{46}O_{46}$ 3577.10 found 3576.8.

Example 7: Preparation of Ac-His-Ser-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Glu-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:9)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Ser, R^{16} is Gln, R^{18} is Glu, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 126 mg (11.4%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{160}H_{256}N_{46}O_{49}$ 3609.06 found 3609.2.

Example 8: Preparation of Ac-His-Ser-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Leu-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:10)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Ser, R^{16} is Gln, R^{18} is Ala, R^{27} is Leu and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 77 mg (7.3%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{158}H_{253}N_{45}O_{47}$ 3536.00 found 3534.95.

Example 9: Preparation of Ac-His-Ser-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Lys-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:11)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Ser, R^{16} is Gln, R^{18} is Lys, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 79 mg (7.5%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{161}H_{261}N_{47}O_{47}$ 3608.11 found 3607.6.

Example 10: Preparation of Ac-His-Ser-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Ala-Nle-Glu-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:12)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Ser, R^{16} is Ala, R^{18} is Glu, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 65 mg (6%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{158}H_{253}N_{45}O_{48}$ 3552.00 found 3551.2.

Example 11: Preparation of Ac-His-Ser-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Leu-Asn-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:13)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Ser, R^{16} is Gln, R^{18} is Ala, R^{27} is Leu and R^{28} is Asn

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 109 mg (10.6%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{156}H_{247}N_{45}O_{48}$ 3521.93 found 3520.5.

Example 12: Preparation of Ac-His-Ala-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:14)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ala, R^5 is Ser, R^{16} is Gln, R^{18} is Ala, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 20 mg (1.8%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{158}H_{254}N_{46}O_{46}$ 3535.02 found 3533.4.

Example 13: Preparation of Ac-His-Ser-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Arg-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:15)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Ser, R^{16} is Arg, R^{18} is Ala, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 60 mg (5.3%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{159}H_{258}N_{48}O_{46}$ 3579.08 found 3577.8.

Example 14: Preparation of Ac-His-Ser-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Arg-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Leu-Asn-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:16)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Ser, R^{16} is Arg, R^{18} is Ala, R^{27} is Leu and R^{28} is Asn

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 40 mg (3.7%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{157}H_{251}N_{47}O_{47}$ 3549.99 found 3549.2.

Example 15: Preparation of Ac-His-Ser-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Arg-Nle-Glu-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:17)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Ser, R^{16} is Arg, R^{18} is Glu, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 36 mg (3.6%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{161}H_{260}N_{48}O_{48}$ 3637.11 found 3636.4.

Example 16: Preparation of Ac-His-Ser-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Arg-Nle-Lys-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:18)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Ser, R^{16} is Arg, R^{18} is Lys, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 51 mg (4.4%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{162}H_{265}N_{49}O_{46}$ 3636.17 found 3634.8.

Example 17: Preparation of Ac-His-Ala-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Arg-Nle-Glu-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:19)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ala, R^5 is Ser, R^{16} is Arg, R^{18} is Glu, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 27 mg (2.7%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{161}H_{260}N_{48}O_{47}$ 3621.11 found 3620.4.

Example 18: Preparation of Ac-His-Ala-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Arg-Nle-Lys-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:20)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ala, R^5 is Ser, R^{16} is Arg, R^{18} is Lys, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 53.5 mg (4.6%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{162}H_{265}N_{49}O_{45}$ 3620.17 found 3618.8.

Example 19: Preparation of Ac-His-Ser-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Arg-Nle-Glu-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Leu-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:21)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is Ser, R^{16} is Arg, R^{18} is Glu, R^{27} is Leu and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 33 mg (3.3%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{161}H_{259}N_{47}O_{48}$ 3622.10 found 3620.8.

Example 20: Preparation of Ac-His-Ser-Asp-Ala-MeVal-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Leu-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:22)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is MeVal, R^{16} is Gln, R^{18} is Ala, R^{27} is Leu and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 55 mg (5.2%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{161}H_{259}N_{45}O_{46}$ 3562.09 found 3561.09.

Example 21: Preparation of Ac-His-Ala-Asp-Ala-MeVal-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:23)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ala, R^5 is MeVal, R^{16} is Gln, R^{18} is Ala, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 49 mg (4.5%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{161}H_{260}N_{46}O_{45}$ 3561.10 found 3560.0.

Example 22: Preparation of Ac-His-Ser-Asp-Ala-MeVal-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Arg-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:24)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ser, R^5 is MeVal, R^{16} is Arg, R^{18} is Ala, R^{27} is Lys and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 13.8 mg (1.2%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{162}H_{264}N_{48}O_{45}$ 3605.16 found 3604.0.

Example 23: Preparation of Ac-His-Ala-Asp-Ala-MeVal-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Leu-Lys-Gly-Gly-Thr-NH_2 [Ac-(SEQ ID NO:25)-NH_2], i.e. compound of formula I wherein X is Ac, Y is NH_2, R^2 is Ala, R^5 is MeVal, R^{16} is Gln, R^{18} is Ala, R^{27} is Leu and R^{28} is Lys

Fmoc-Rink-Linker-BHA resin (450 mg, 0.25 mmol) was subjected to solid phase synthesis and purification by following the procedure in Example 1 to yield 30.2 mg (2.8%) of white amorphous powder. (ES)+-LCMS m/e calcd for $C_{161}H_{259}N_{47}O_{45}$ 3546.09 found 3544.8.

Example 24: Sup-T1 cAMP Agonist Assay

The human T-lymphoid cell line Sup-T1, which expresses the VPAC2 receptor, was obtained from the American Type Culture Collection (ATCC, CRL-1942) and maintained in growth medium at densities between 0.2 and 2 x 10^6 cells/ml in a 37°C CO_2 incubator. The growth medium was RPMI 1640 (Invitrogen) supplemented with 25 mM HEPES buffer and 10% fetal bovine serum (Gemini Bioproducts).

To evaluate VPAC2 agonist compound activity, cells in log-phase growth were washed once with growth medium at RT and plated into 96-well plates at a density of 4 x 10^4 cells per well in 150 µl of growth medium. 50 µl of the compounds to be tested, prepared at appropriate concentrations in growth medium, were then added to designated wells. After 5 min at RT, the cells were lysed by adding 25 µl of lysis reagent 1A (cAMP Biotrak EIA system, Amersham Biosciences, RPN225) to each well. The 96-well plates were kept at RT for 10 min with shaking and then stored at 4°C until analysis for cAMP (within 2 hr).

Cyclic AMP levels were determined in 100 µl of each lysate using the cAMP Biotrak Enzyme immunoassay (EIA) kit according to the manufacture's instructions (Amersham Biosciences, RPN225). The activity of each VPAC2 agonist compound (EC_{50} value) was estimated by fitting the 7-concentration dose response data to a sigmoidal dose-response equation provided by the GraphPad Prism program (GraphPad Software, Inc.).

Table 1

Compound in Example	Sup-T1 cAMP EC50 (nM)
1	38
2	69.7
3	98
4	85
5	1206
6	2.35
7	632
8	11.4
9	1200
10	447
11	16.8
12	7.4
13	17.6
14	94.1

15	116.7
16	1030
17	23.3
18	276
19	68.4
20	9.45
21	4.75
22	10.54
23	4.07

Example 25: Peptide Stability to Neutrophil Elastase

The proteolytic stabilities of peptide analogs were established with reversed phase high pressure liquid chromatography (RP HPLC) electrospray ionization mass spectrometry (ESI MS). Peptide analogs were incubated with human neutrophile elastase and the quantity of undigested analogs was determined by ESI MS at appropriate time points. Multiple peptide analogs could be included in one experiment as long as they could be differentiated by HPLC retention time and/or by molecular weight. Ac-His Ac-His-Ser-Asp-Ala-Val-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH_2 was used in all experiments as a control and as a reference standard. The simultaneous use of multiple peptide analogs together with a reference standard allowed for compensation for variations in the proteolytic fidelity of the enzyme over the multiple experiments. Integrated ion currents obtained for the individual undigested peptide were used for quantitation. For calculation of halftime first-order kinetic behavior was assumed and all calculations were normalized to the halftime of the reference standard.

Peptide stock solutions were prepared in water to a concentration of 2.5 mg/mL. Unless in use, all stock solutions were kept at -20°C. In order to determine the relative peptide content in the prepared stock solutions reversed phase HPLC was done with an aliquot and the observed UV absorbance was compared with a comparable aliquot from the reference standard. Concentrations of the peptide analogs were adjusted accordingly. In order to do the proteolytic digestion, peptides were dissolved in phosphate buffered saline (PBS) to a concentration of 0.1 mg/mL. As many as six different peptide analogs were mixed into one 50 µL reaction volume. The reference standard was added to all experiments as a reference and internal standard. Elastase (Human Neutrophil, Calbiochem, Cat # 324681) was added from an elastase stock solution to a concentration of 1 to 2 µg/mL. Different amounts of

the enzyme were chosen to compensate for the differences in the proteolytic stabilities of the peptide analogs. Previously, a stock solution of elastase was prepared in water at a concentration of 1 mg/mL. Small aliquots of the enzyme stock solution were kept at -20°C to better maintain the enzyme activity by limiting the number of thaw and freeze cycles. The digestion was done at ambient temperature in an autosampler tube within the autosampler of the HPLC system (Agilent 1100 Series). For a time course, 5 µL aliquots were injected in 70 min intervals onto the reversed phase HPLC column (Phenomenex, Luna C18, 3µ, 100Å, 150 x 2.00 mm). For the starting time point an aliquot was injected just prior to the addition of the proteolytic enzyme. A total of eight time points could be recorded from one experiment, including the starting point. Peptides were separated on the reversed phase column with a 50 min gradient of 5 % to 30 % organic phase. The aqueous phase was 0.05 % (v/v) of trifluoroacetic acid in water and the organic one was 0.045 % (v/v) of trifluoroacetic acid in acetonitrile. Absorbances were recorded at 214 and 280 nm respectively. All of the column effluent was introduced into the turbo V source of the electrospray ionization mass spectrometer (ABI 4000 QTrap LC/MS/MS System). Mass spectra were acquired in Q3MS mode in a mass range to include all triply charged ions of the non degraded peptide analogs. Care was taken to assure that peptide analogs could clearly be differentiated either by the chromatographic retention time or by the difference in molecular weight. Relative quantities of the respective undigested peptide analog were calculated from the integrated total ion current. A window of 2.5 Da was chosen and the manufacturer's software was used to integrate the individual ion currents. The overall halftime of an individual peptide analog was calculated by assuming first-order kinetic behavior and was normalized with respect to the halftime of the reference standard.

Table 2

Compound in Example	Relative elastase stability
1	3.9
2	5.2
3	4.5
4	3.9
5	4.9
6	6
7	16.4
8	3.5
9	12.0
10	7.4

11	2.4
12	5.2
13	1.7
14	4.5
15	4.8
16	4.4
17	4.6
18	5.1
19	3.3
20	3.4
21	5.7
22	1.8
23	3.6

Example 26: Effect of Compounds on LPS-Induced Lung Inflammation in Male C57BL/6 Mice

Aerosol LPS: C57bl/6 mice are pretreated with vehicle or drug prior to an aerosol expose to lipopolysacchride (LPS, 500 µg/ml in sterile saline) for 15-30 minutes. The aerosol is generated by a Pari Ultra neb jet nebulizer, the outlet of which is connected to a small clear plastic chamber [H x W x D, 10.7 x 25.7 x 11 cm (4 x 10 x 4.5 in)] containing the animals. Bronchoalveolar lavage (BAL) is performed 24 hr later to determine the intensity of cell inflammation. BAL procedure is performed as described below.

Intranasal administration of LPS: Mice are pretreated with vehicle or drug prior to an intranasal administration of lipopolysacchride (0.05-0.3 mg/kg in sterile saline; 50 µl total volume, 25 µl/nostril). Intranasal administration is performed by presenting small droplets of the dosing solution at the nostril using a 25-50 µl eppendorff pipet. BAL is performed 3 to 24 h post LPS challenged as described above to determine the intensity of cell inflammation.

Bronchoalveolar lavage: 24 h following LPS exposure, animals are anesthetized with pentobarbital (80-100 mg/kg, i.p.), ketamine/xyzaline (80-120 mg/kg/2-4 mg/kg, i.p.) or urethane (1.5 - 2.4 g/kg, i.p.); and through a small midline neck incision (15-20 mm), the trachea is exposed and cannulated with 20-gauge tubing adapter. Lungs are lavaged with 2 x 1 ml sterile Hank's balanced salt solution without Ca++ and Mg++ (HBSS). Lavage fluid is recovered after 30 sec by gentle aspiration and pooled for each animal. Samples are then centrifuged at 2000 rpm for 10 min at 5°C . Supernatant is aspirated, and red blood cells

are lysed from the resulting pellet with 0.5 ml distilled water for 30 sec before restoring osmolarity to the remaining cells by the addition of 5 ml of HBSS. Samples are recentrifuged at 2000 rpm for 10 min at 5°C and supernatant aspirated. The resulting pellet is resuspended in 1 ml of HBSS. Total cell number is determined by Trypan Blue (Sigma Chemical, St. Louis, MO) exclusion from an aliquot of cell suspension using a hemocytometer or coulter counter. For differential cell counts, an aliquot of the cell suspension is centrifuged in a Cytospin (5 min, 1300 rpm; Shandon Southern Instruments, Sewickley, PA) and the slides fixed and stained with a modified Wright's stain (Hema 3 stain kit, Fisher Scientific). Standard morphological criteria is used in classifying at least 300 cells under light microscopy. Data in Table 3 is expressed as BAL cells x 10^4/animal for neutrophils and total cells, or percent inhibition of the LPS induced BAL fluid neutrophilia response.

Table 3

Compound in Example	Dose	Inhibition of LPS-induced neutrophilia (+ 10-30%, ++ >30%)
1	0.1%	+
2	0.1%	+
6	0.01%	++
7	0.01%	++
8	0.1%	++
9	0.01%	+
10	0.01%	++
11	0.01%	++
12	0.01%	+
13	0.01%	++
15	0.01%	+
17	0.01%	+
19	0.01%	+

Example 27: Effect of Compounds on Methacholine-Induced Bronchospasm in Mice

Respiratory function is measured in conscious, freely moving mice using whole body plethysmographs (WBP) from BUXCO Electronics, Inc. (Troy, NY). WBP chambers allow animals to move freely within the chamber while respiratory function is measured. Eight chambers are used simultaneously so that eight mice can be measured at the same time.

Each WBP chamber is connected to a bias flow regulator to supply a smooth, constant flow of fresh air during testing. A transducer attached to each chamber detects pressure changes that occur as the animal breathes. Pressure signals are amplified by a MAX II Strain Gauge preamplifier and analyzed by the Biosystem XA software supplied with the system (BUXCO Electronics, Inc.). Pressure changes within each chamber are calibrated prior to testing by injecting exactly 1 ml of air through the injection port and adjusting the computer signal accordingly. Mice are placed in the WBP chambers and allowed to acclimate for 10 min prior to testing. Testing is conducted by letting the animals move and breathe freely for 15 min while the following parameters are measured: Tidal Volume (ml), Respiratory Rate (breaths per min), Minute Volume (tidal volume multiplied by respiratory rate, ml/min), Inspiratory Time (sec), Expiratory Time (sec), Peak Inspiratory Flow (ml/sec), and Peak Expiratory Flow (ml/sec). Raw data for each of the parameters listed above are captured in the software database and averaged once per min to give a total of 15 data points per parameter. The average of the 15 data points is reported. Accumulated Volume (ml) is a cumulative value (not averaged) and represents the sum of all tidal volumes for the 15 min test session.

The protocol is customized to include measurements before, during, and after a spasmogen challenge to determine Penh. Dose-response effects of a particular spasmogen (i.e. methacholine (MCh), acetylcholine, etc.) are obtained by giving nebulized aerosol (30-60 sec exposure) at approximately 5 - 10 min intervals.

Mice (balb/c) are treated with vehicle (2% DMSO in H_2O) or drug dissolved in 4 ml vehicle for 20 minutes by aerosol, as described above, prior to spasmogen challenge. Penh is determined at 5, 30 and 60 minutes post-challenge. Data are reported as percent inhibition of Penh relative to vehicle.

Table 4

Compound in Example	Inhibition of Penh at 5 min post-challenge (+ >50%, ++ <50%)
6	++
12	+
18	+
19	+

Claims:

1. A cyclic vasoactive intestinal peptide analog of the formula I

X-His-R^2-Asp-Ala-R^5-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-R^{16}-Nle-R^{18}-Ala-Lys-Lys^{21}-Tyr-Leu-Asn-Asp^{25}-Leu-R^{27}-R^{28}-Gly-Gly-Thr-Y

[X-(SEQ ID NO: 2)-Y]

wherein

X is a hydrogen of the N-terminal amino of Histidine which may be optionally replaced by a hydrolyzable amino protecting group, most preferably by an acetyl group,

Y is the hydroxy of the C-terminal carboxy of Threonine which may be optionally replaced by a hydrolyzable carboxy protecting group, most preferably by NH_2,

underlined residues indicates a side-chain to side-chain covalent linkage of the first (Lys^{21}) and last (Asp^{25})amino acids within the segment,

R^2 is Ser or Ala,

R^5 is Thr, Ser, Asp, Gln, Pro or CαMeVal,

R^{16} is Gln, Ala, or Arg,

R^{18} is Ala, Lys or Glu,

R^{27} is Lys or Leu except that R^{27} must be Lys when R^5 is CαMeVal and R^{16} is Arg,

R^{28} is Lys or Asn,

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1 wherein R^5 is Ser or CαMeVal.

3. The compound of claim 2 wherein R^{27} is Lys.

4. A compound selected from the group consisting of

His-Ser-Asp-Ala-Thr-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr (SEQ ID NO: 3),

His-Ser-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr (SEQ ID NO: 4),

His-Ser-Asp-Ala-MeVal-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr (SEQ ID NO: 8),

His-Ser-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Lys-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr (SEQ ID NO: 11),

His-Ala-Asp-Ala-Ser-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Arg-Nle-Glu-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr (SEQ ID NO: 19),

His-Ala-Asp-Ala-MeVal-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr (SEQ ID NO: 23), and His-Ala-Asp-Ala-MeVal-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Leu-Lys-Gly-Gly-Thr (SEQ ID NO: 25).

5. The compound of claim 1 which is X-(SEQ ID NO: 8)-Y.

6. A pharmaceutical composition comprising a compound of claim 1 and at least one pharmaceutically acceptable carrier or excipient.

7. A method for treating pulmonary obstructive disorders comprising administering by inhalation an effective amount of a composition comprising a compound of claim 1 and at least one pharmaceutically acceptable carrier or excipient to a person suffering from such disorder.

8. The use of a compound of claim 1 for the preparation of a medicament for treating pulmonary obstructive disorders.

9. A process for the preparation of a compound of claim 1.

10. The invention as hereinbefore described.